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Modification of the isotopic composition of rainfall by throughfall and stemflow: the case of Scots pine and Downy oak forests under Mediterranean conditions

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Short title: Isotopic modification of rainfall by canopy interception processes

ABSTRACT

Most hydrological studies based on stable water isotopes (^{18}O and D) use the isotopic composition of rainfall as input signal. Although stable water isotopes are conservative tracers, previous studies have shown that canopies modify the isotopic composition of rainfall. At present, there is scientific agreement about the factors involved in isotopic modification, but the effect of each factor and the magnitude of the isotopic shift are still not clear. In this study, we analyse at an inter- and intra-event basis the spatio-temporal differences between the isotopic composition of rainfall, throughfall and stemflow for two different species (*Pinus sylvestris* L. and *Quercus pubescens* Willd). The aim of the study is to analyse the isotopic modification that takes place in throughfall and stemflow and how meteorological variables and structural forest characteristics influence the observed changes. Rainfall and throughfall were sampled by a combination of bulk and sequential collectors, whereas stemflow was collected only by bulk collectors. Results showed that the isotopic modification occurred in both directions, although stemflow was consistently more enriched than throughfall. Despite the contrasting canopy structures, no significant differences between species were found. Moreover, the intra-event analysis suggested that all fractionation factors could occur during one event,

but evaporation or isotopic exchange would have a higher impact at the beginning of rainfall, whereas canopy selection processes would be more important at the end of rainfall. Our results emphasise the importance of considering the isotopic composition of throughfall and stemflow in isotope-related studies in forested catchments.

Key words: Stable isotopes, Canopy interception, Rainfall partitioning, Throughfall, Stemflow, Spatio-temporal variability, Vallcebre research catchments.

1. INTRODUCTION

In recent decades, the isotopic composition of rainfall (^{18}O and D) has increasingly been used as an input signal to trace the source and movement of water in a catchment (Kendall & McDonnell, 1998). However, in forested or partly forested catchments, throughfall and stemflow have seldom been considered when defining the catchment input signal, although previous studies have shown that there may be a shift in their isotopic composition.

Saxena (1986) was one of the first to observe that throughfall was in general more enriched in heavy isotopes of Oxygen ($\delta^{18}O$) than open rainfall, even though depletion was also found on some occasions. Enrichment was attributed to isotopic fractionation in non-equilibrium conditions, whereas depletion was associated with the retention in the canopy of the last portion of rain during events of varying isotopic composition. This process was named selective canopy storage by Dewalle & Swistock (1994). In addition, these authors, noting the lack of relationship between interception loss and the isotopic composition of throughfall, and also because samples fell on the local meteoric water line, suggested that selective canopy storage was more important than fractionation caused by evaporation. Friedman (1962) also showed that isotopic fractionation could be achieved by the isotopic exchange between vapour and liquid during high-humidity atmosphere conditions. Molecular exchange could result in enrichment or depletion, preferably enrichment, except under conditions of relative humidity close to 100% and a high difference in $\delta^{18}O$ between rain water and water vapour (Brodersen, Pohl, Lindenlaub, Leibundgut, & Wilpert, 2000). More recently, Allen, Brooks, Keim, Bond, & McDonnell (2014) suggested that the isotopic composition of throughfall could also be influenced by the presence of residual water from previous rainfall, retained within the canopy and mixed with the new rainfall input, resulting in either enrichment or depletion. However, their results were for a location with high mean annual precipitation (2000 mm year^{-1}), high mean relative humidity (99%) and inter-event rain-free periods shorter than 2 days.

The isotopic composition of stemflow is generally assumed to undergo similar processes as throughfall. However, Kubota & Tsuboyama (2003) observed that stemflow samples were in general more enriched in $\delta^{18}O$ than throughfall samples, although no specific reasons for such differences were discussed by the authors. Ikawa, Yamamoto, Shimada, & Shimizu (2011), based on the different isotopic dynamics of stemflow rather than open rainfall and throughfall, suggested that the isotopic composition of stemflow could be affected more by mixing with rain water previously stored in the canopy and the stems. All these processes occur in the canopy and result in isotopic offsets of throughfall and stemflow from rainfall. Offsets can be different depending on the canopy characteristics, usually being greater in coniferous forests than in broadleaf forests, possible due to their higher storage capacity (Allen, Keim, Barnard, McDonnell, & Brooks, 2017). Until now, research efforts have tried to understand the factors that produce the modification of the isotopic composition of water that falls through the canopy, but no clear temporal or spatial patterns have yet been found (Allen et al., 2017). Moreover, most recent studies have focused on throughfall (i.e. Allen et al., 2014; Allen, Keim, & McDonnell, 2015; Brodersen et al., 2000; Hsueh, Allen, &

Keim, 2016; Kato et al., 2013; Qu et al., 2014; Xu, Guan, & Deng, 2014), whereas shifts in the isotopic composition of stemflow have been much less widely studied (i.e. Ikawa et al., 2011; Kubota & Tsuboyama, 2003) despite recent studies have highlighted its importance as a preferential flow-path of water to the soil (Levia & Germer, 2015). In the study area, stemflow accounted for ~1% of the incident rainfall; however, stemflow reaching the base of a tree (expressed as $l\ m^{-2}$) could represent more than 10 times the volume of rainfall, therefore, its influence on the isotopic composition of soil water should not be underestimated (Cayuela, Llorens, Sánchez-Costa, Levia, & Latron, 2018).

The analysis of the intra-event variability of the isotopic composition of throughfall and stemflow has proved to be a useful tool (Allen et al., 2017), although there are only a few studies (Ikawa et al., 2011; Kubota & Tsuboyama, 2003; Qu et al., 2014) and these have no strong concluding remarks. Therefore, there is still an important challenge to understand how and why the isotopic composition of rain is modified during rainfall partitioning processes (Allen et al., 2017; Hsueh et al., 2016) and what implications this has for the identification of water sources and paths through a forested or partly forested catchment. Low resolution samplings of soil water (weekly or monthly) may dampen the propagation of any interception effect in the soil (Stockinger et al., 2016) and reduce the isotopic spatial variability of soil water. Despite this fact, Stockinger et al. (2015) found that changes in the isotopic composition of open rainfall due to canopy interception were relevant and had to be considered for isotope-based transit time studies. In addition, other studies using higher sampling resolution (event sampling) like Kubota & Tsuboyama (2003) also found differences when incorporating the isotopic composition of throughfall; in that case they found differences of 5-10% in the contribution of pre-event water for hydrograph separation. These studies show up the importance of considering throughfall and stemflow in hydrological studies.

In this study, we examine the paired isotopic differences of throughfall-rainfall and stemflow-rainfall, using a combination of bulk and sequential samples. The main objectives of the study are (i) to analyse the spatio-temporal differences between the isotopic composition of rainfall, throughfall and stemflow for two different species: *Pinus sylvestris* L. (Scots pine) and *Quercus pubescens* Willd. (downy oak) and (ii) to relate these differences to different meteorological conditions and structural forest characteristics to gain some knowledge on the fractionation factors that occur in the canopy.

2. METHODOLOGY

2.1. Study area

The study area is located in the Vallcebre research catchments (NE Spain, 42º 12'N, 1º 49'E) in the eastern Pyrenees at 1100 m asl (meters above sea level). These catchments have been monitored for various hydrological purposes since 1988 (Llorens et al., 2018). The climate is Sub-Mediterranean, with a mean annual temperature of 9.1 ± 0.67ºC, a mean annual precipitation of 880 ± 200 mm and a mean annual evapotranspiration of 823 ± 26 mm (1989-2015). The precipitation regime is seasonal; autumn and spring are usually wetter, while summer and especially winter are often dryer seasons. Summer rainfall is characterized by intense convective events, whereas during the rest of the year precipitation is generally caused by frontal systems.

The original oak forest (*Quercus pubescens* Willd.), in the sunny aspects, and Scots pine (*Pinus sylvestris* L.), in the shady ones, were deforested in the past and most of the area was terraced for agricultural production. After the abandonment of agricultural activities in the sixties, most of the terraces underwent spontaneous afforestation by

Scots pines (Poyatos, Latron, & Llorens, 2003). Two forest plots were selected for the study, a pine and an oak stand. The pine stand is oriented towards the northeast at an elevation of 1200 m and has an area of 900 m^2, a tree density of 1189 trees ha^{-1} and a basal area of 45.1 m^2 ha^{-1}. The oak stand is oriented towards the southeast at an elevation of 1100m and has an area of 2200 m^2, a tree density of 518 trees ha^{-1} and a basal area of 20.1 m^2 ha^{-1} (Figure 1).

2.2. Hydrometric and meteorological monitoring

In each stand, rainfall was measured with a tipping bucket rain gauge located in a clearing less than 100 m from each stand. Throughfall was measured with 20 tipping bucket rain gauges (Davis Rain Collector II, Davis Instruments) spatially distributed according to canopy cover distribution. The tipping buckets were placed at the 20 most representative locations. Canopy cover was determined from 50 hemispherical photographs taken at each stand. A complete description of the method to determine the canopy cover can be found in Llorens & Gallart (2000). Stemflow was measured in seven trees, representing the range of diameter at breast height (DBH) distributions, with stemflow rings connected to tipping bucket rain gauges. Meteorological data were obtained from 15 and 18 m towers at the oak and pine stands, respectively. Each station monitored air temperature, relative humidity, net radiation, wind speed and wind direction 1 m above the canopy. Wet canopy evaporation was calculated by the Penman–Monteith equation with a stomatal resistance set to zero (Stewart, 1977). All data were recorded at 5-min intervals by a datalogger (DT80, Datataker Inc.).

2.3. Isotopic sampling

Sampling was carried out from May 2015 to May 2016 on an event basis. To take into account seasonal changes in canopy cover, as well as possible temporal differences due to air temperature, two time-periods were considered: the growing season from May

15th to October 15th, which covered the period of higher air temperature; and the dormant season for the remaining months, which covered the period of lower temperature. To ensure the dryness of the canopy between successive rainfall events, the inter-event period was set to be at least 6 hours (without any rainfall) during the day and 12 hours during the night (Llorens, Domingo, Garcia-Estringana, Muzylo, & Gallart, 2014). As a result, 22 individual rainfall events that had not been mixed with previous or following events, were analysed.

In each study plot, throughfall was sampled with 10 collectors consisting of plastic funnels 130 mm in diameter positioned 50 cm above ground and connected to a plastic bin by looped tubing. The plastic bin had 1 litre capacity and was placed in the ground to prevent heating and evaporation. The location of each throughfall collector was selected to represent all ranges of canopy cover in each stand (from 30 to 88% in the pine stand, and from 30 to 95% in the oak stand). In addition, throughfall was sampled automatically, at 5 mm rainfall intervals, using a plastic funnel (340 mm diameter) connected to an automatic water sampler (ISCO 3700C). Stemflow was sampled on 4 trees with different DBH (~ 15, 20, 25 and 30 cm) representative of the DBH distributions in each stand, using a stemflow ring connected to a 60 litre polyethylene bin by looped tubing. Rainfall was sampled in a clearing near each stand by means of a bulk collector and an automatic sampler (5 mm rainfall intervals) in the same way as for throughfall.

To ensure the reliability of the collectors in preventing evaporation, one additional collector was filled with water of a known isotopic composition and was sampled once a week. After 5 weeks, water in this collector showed a mean fractionation of 0.05‰ for $\delta^{18}O$ and 0.30‰ for δD. Nonetheless, all samples used in this study were collected within 1 to 4 days after each storm; and funnels and bins were cleaned and dried before the following rainfall.

2.4. Isotopic analysis

Stable water isotopes (^{18}O and D) were analysed by a Cavity Ring-Down Spectroscopy Picarro L2120-i isotopic water analyser at the Scientific and Technological Services of the University of Lleida. Accuracy of the L2120-i, based on the repeated analysis of four reference water samples, was < 0.1‰ and < 0.4‰ for $\delta^{18}O$ and δD, respectively. All isotope data are expressed in terms of δ-notation as parts per mil (‰). Moreover, deuterium excess (d-excess) which relates δD and $\delta^{18}O$ (Equation 1) was calculated and used as an indicator of kinetic or equilibrium fractionation (Dansgaard, 1964).

$$d\text{-}excess = \delta D - 8 \cdot \delta^{18}O \quad (1)$$

2.5. Data analysis

The isotopic modification of throughfall ($\Delta\delta^{18}O_{TF\text{-}RF}$) was calculated as the difference between $\delta^{18}O$ of throughfall and $\delta^{18}O$ of rainfall; and the isotopic modification of stemflow ($\Delta\delta^{18}O_{SF\text{-}RF}$), as the difference between $\delta^{18}O$ of stemflow and $\delta^{18}O$ of rainfall. The modification of the d-excess of throughfall (Δd-excess$_{TF\text{-}RF}$) and stemflow (Δd-excess$_{SF\text{-}RF}$) was expressed similarly. The combination of the isotopic and d-excess differences was used to speculate about the operating mechanisms in the canopy (Brodersen et al., 2000). To analyse the isotopic modification of throughfall and stemflow at the event scale, a linear mixed model (LMM) with repeated measurement structure was set. After checking for collinearity among measured variables, the model included rainfall depth, maximum wind speed, canopy cover, DBH, season and species, as fixed factors; and the location of each collector, as a random effect. Results of the model are expressed according to the Fisher distribution ($F_{\text{dfn, dfd}}$), indicating the degrees of freedom in the numerator (dfn) and degrees of freedom in the denominator (dfd).

Finally, to analyse possible temporal persistent stability patterns of throughfall depth, $\Delta\delta^{18}O$ and Δd-excess, time-stability plots (Keim, Skaugset, & Weiler, 2005) with standardized data were performed.

3. RESULTS

4. Isotopic composition of rainfall, throughfall and stemflow

The rainfall depth of the analysed events ranged from 2.3 mm to 69.1 mm; and mean rainfall intensities, from 0.4 mm h^{-1} to 23.0 mm h^{-1}. Overall, mean rainfall intensity increased with rainfall depth ($F_{1, 428} = 566.6$; $p < 0.01$). For these events, mean relative throughfall was 77% in the pine stand and 76% in the oak stand. Mean relative stemflow accounted for 1.5% and 0.9% of the incident rainfall in the pine and oak stands; four events did not produce enough stemflow to measure its isotopic composition.

$\delta^{18}O$ values in bulk rainfall of both stands ranged from -12.32‰ to -1.72‰; and δD values, from -92.30‰ to -4.18‰. Rainfall samples fell on the Local Meteoric Water Line (LMWL) of the Vallcebre Research Catchments, $\delta D = 7.96\ \delta^{18}O + 12.89$, which was determined by the least squares method for $\delta^{18}O$ and δD measured in bulk rainfall samples during the period 2011-2016. Throughfall and stemflow samples also fell on the LMWL; values of $\delta^{18}O$ of throughfall in the pine stand ranged from -12.13‰ to -2.03‰ and in the oak stand from -11.33‰ to -1.83‰. For stemflow, $\delta^{18}O$ values ranged from -10.61‰ to -2.33‰ in the pine stand and from -10.46‰ to -1.22‰ in the oak stand (Figure 2). In general, the isotopic composition of throughfall and stemflow followed similar distribution to rainfall but with heavier isotopic composition for $\delta^{18}O$ ($F_{2, 26} = 129.24$; $p < 0.01$) (Figure 3 a and b) and for δD ($F_{2, 26} = 90.74$; $p < 0.01$) (Figure 3 c and d). In general, for both isotopes, throughfall was more enriched than rainfall and stemflow was more enriched than throughfall. In the pine stand, 55% of throughfall and

81% of stemflow samples were enriched in $\delta^{18}O$. In the oak stand, enrichment occurred for 50% of throughfall and 94% of stemflow samples. Similar trends were observed for δD.

Between stands, however, there were no statistically significant differences in $\Delta\delta^{18}O_{TF-RF}$ ($F_{1, 16} = 2.91$; $p = 0.11$) and $\Delta\delta^{18}O_{SF-RF}$ ($F_{1, 4} = 1.53$; $p = 0.28$); the $\Delta\delta^{18}O_{TF-RF}$ in the pine stand ranged between -1.13‰ and 2.05‰, and in the oak stand between -1.02‰ and 1.25‰. On the other hand, $\Delta\delta^{18}O_{SF-RF}$ ranged between -2.1‰ and 3.08‰ in the pine stand, and between -0.52‰ and 3.07‰ in the oak stand. From Figure 3e and f, it can be inferred that not all samples enriched in $\delta^{18}O$ and δD corresponded with a decrease of d-excess and not all depleted samples in $\delta^{18}O$ and δD corresponded with an increase of d-excess, as would be expected from non-equilibrium fractionation processes. Indeed, from the enriched samples in the Scots pine stand, only 35% of throughfall and 37% of stemflow samples had negative d-excess. In the oak stand, this was the case for 28% of throughfall and 51% of stemflow samples; similar percentages were found for δD.

4.1. Spatio-temporal patterns in the modification of the isotopic composition of rainfall

Rainfall with heavier $\delta^{18}O$ was more common in events occurring at the end of spring and in summer, when air temperature was higher. In general, a seasonal pattern linked to air temperature was observed in the isotopic composition of rainfall throughout the year (Figure 4a and b). Results showed that depleted throughfall (negative $\Delta\delta^{18}O_{TF-RF}$) was more common during the growing season ($F_{1, 404} = 4.39$; $p < 0.05$) (Figure 4c and d). Moreover, $\Delta\delta^{18}O_{TF-RF}$ decreased for rainfall depths higher than 20 mm ($F_{1, 404} = 4.22$; $p < 0.05$) in both stands (Figure 5a). For rainfall depths lower than 20 mm (corresponding with rainfall intensities lower than 5 mmh^{-1}), differences between throughfall and rainfall were higher. In addition, a greater spatial variability between throughfall collectors was observed during these events, with $\delta^{18}O$ coefficients of variation (CV) up to 10% in the pine stand and 15% in the oak stand. For higher rainfall depth (>20 mm), CV were in general lower than 5% (Figure 5b). On the other hand, stemflow was more enriched (positive $\Delta\delta^{18}O_{SF-RF}$) during the growing season ($F_{1, 127}$ = 13.13; $p < 0.01$) (Figure 4e and f) and isotopic differences were marginally less for higher rainfall amounts ($F_{1, 127}$ = 3.02; $p = 0.08$) (Figure 5c). The spatial variability of $\delta^{18}O$ among collectors was also higher for low rainfall amounts, with CV up to 20% in the oak stand and 30% in the pine stand. Higher rainfall amounts decreased CV among collectors (Figure 5d).

The spatial distribution of throughfall depth measured in each collector from event to event showed a persistent temporal stability (Figure 6a) that neither $\Delta\delta^{18}O_{TF-RF}$ (Figure 6b) nor Δd-ecxess$_{TF-RF}$ (Figure 6c) had in pines or oaks. However, a marginal relationship ($F_{1, 16}$ = 3. 52; $p = 0.07$) between the $\Delta\delta^{18}O_{TF-RF}$ and the canopy cover was found (Figure 6e). This effect was more clearly seen in the pine stand, where the most covered collectors had higher $\Delta\delta^{18}O_{TF-RF}$. However, throughfall volume and Δd-excess$_{TF-RF}$ did not show persistent temporal stability patterns (Figures 6d and 6f) when ranked by canopy cover. No stable patterns could be observed for throughfall in oaks during the dormant season neither for stemflow (data not shown).

4.2. Intra-storm isotopic composition of rainfall and throughfall

The intra-storm isotopic modification of rainfall was analysed for 10 events that were sequentially sampled. Mean rainfall depth for those events was 29.8 mm in the pine stand and 27.2 mm in the oak stand, and ranged between 10 mm and 67 mm. From the 10 analysed events, seven corresponded to the growing season, thus the dormant season was less represented in the analysis. Selected events were divided into three stages of the storm: initial, representing the first 5 mm of each event; middle, representing all samples between the first and the last sample; and final, representing the last sample collected. Each consecutive stage had a statistically significant difference in the d-excess of rainfall ($F_{2, 61} = 3.98$; $p < 0.05$) and throughfall ($F_{2, 52} = 3.94$; $p < 0.05$).

Results showed similar trends between forest stands. In general, the isotopic compositions of rainfall and throughfall were heavier at the beginning of the event (Figures 7c and e). These heavier values coincided with the highest values of vapour pressure deficit (VPD) and intercepted rainfall (difference between rainfall and throughfall) (Figures 7a and b). d-excess increased during the middle stage and decreased during the final stage (Figures 7d and f). The $\Delta\delta^{18}O_{TF\text{-}RF}$ was also higher at the beginning of the event (mean $\delta^{18}O$ difference of 0.32 ± 0.61‰ in pines and 0.27 ± 0.55‰ in oaks) and decreased during the rainfall event (mean $\delta^{18}O$ difference of -0.14 ± 0.86‰ in pines and -0.02 ± 0.86‰ in oaks at the final stage) (Figure 7g). On the contrary, Δd-excess$_{TF\text{-}RF}$ tended to increase during the event (Figure 7h), with mean differences ranging from lower values at the beginning of the event (-0.40 ± 1.62‰ in pines and -1.05 ± 2.61‰ in oaks) to higher values at the end (2.04 ± 2.85‰ in pines and 0.58 ± 2.95‰ in oaks).

For each sequentially sampled event, there was a negative relationship between the mean $\Delta\delta^{18}O_{TF\text{-}RF}$ and the mean Δd-excess$_{TF\text{-}RF}$ at the pine stand ($F_{1, 8} = 54.51$, $p < 0.01$) and at the oak stand ($F_{1, 8} = 9.13$, $p < 0.05$) (Figure 8) (both mean $\delta^{18}O$ and d-excess for each event were weighted by the volume of each sample). According to this relationship, the isotopic dynamic of three different events was analysed: event 8 had negative $\Delta\delta^{18}O_{TF\text{-}RF}$ and positive Δd-excess$_{TF\text{-}RF}$; event 10 had positive $\Delta\delta^{18}O_{TF\text{-}RF}$ and negative Δd-excess$_{TF\text{-}RF}$; and event 12 had differences in $\delta^{18}O$ and d-excess close to zero. Meteorological and isotopic characteristics of each event are shown in Table 1.

The isotopic composition of throughfall showed similar dynamics to the isotopic composition of rainfall. d-excess was always positive during event 8 (Figure 9a), always negative during event 10 (Figure 9b) and variable during event 12 (Figure 9c). Nonetheless, in both stands the dynamics of $\delta^{18}O$ and d-excess were similar. The representation of the sequential samples of rainfall and throughfall in the dual space ($\delta^{18}O$ and δD) showed the space of mixing waters (Figure 10). In this space the isotopic composition of throughfall and rainfall had in general greater differences during the short events. For event 8 (Figure 10a) throughfall was depleted in both stands; however, the distance between mixing spaces was much greater in the Scots pine stand (the mean $\Delta\delta^{18}O_{TF-RF}$ was -0.87‰ for pines and -0.20‰ for oaks). Event 10 (Figure 10b) had enriched throughfall in comparison to rainfall, with mean $\Delta\delta^{18}O_{TF-RF}$ of 1.05‰ in the pines and 1.14‰ in the oaks. For event 12, the mixing spaces overlapped (Figure 10c). Bulk throughfall samples (dots in Figure 10) showed some spatial variability in their isotopic composition. However, these samples were mostly distributed within the throughfall mixing spaces defined by the sequential samples.

5. DISCUSSION

5.1. Temporal variability of the isotopic composition of rainfall, throughfall and stemflow

The isotopic composition of throughfall and stemflow was in general more enriched than that of rainfall. However, all samples fell along the LMWL, indicating that, in general, fractionation happened in both isotopes ($\delta^{18}O$ and δD). The isotopic composition of rainfall showed a seasonal effect related to air temperature. In general, higher $\delta^{18}O$ in rainfall was observed in summer and lower $\delta^{18}O$ in winter. According to Gat (1996), higher $\delta^{18}O$ is consistent with rainfall that contains more water condensed at higher temperature and that evaporates more during its descent.

As observed by others (i.e. Dewalle & Swistock, 1994; Saxena, 1986; Stockinger, Lücke, Vereecken, & Bogena, 2017; Xu et al., 2014), our data also showed an enrichment pattern, with lighter throughfall than rainfall more common during the growing season (at higher temperatures), and heavier throughfall than rainfall more common during the dormant season (at lower temperatures). This pattern corroborates that fractionation is temperature-dependent and that molecular bonds between lighter isotopes are more easily broken than molecular bonds between heavier isotopes (Majoube, 1971).

Positive and negative $\Delta\delta^{18}O_{TF\text{-}RF}$ happened almost in the same proportion; no significant differences were found between stands regardless of the different canopy structures. The highest isotopic differences between throughfall and rainfall, in either direction, were found for events with fewer than 20 mm of rainfall. In that sense, higher enrichment was observed for low rainfall volumes and intensities in a boreal Scots pine forest in northern Scotland (Soulsby, Braun, Sprenger, Weiler, & Tetzlaff, 2017). These events did not completely saturate the canopy, resulting in a non-uniformly wet canopy that might have increased the variability of throughfall amount and also of its isotopic composition. In some locations below the canopy, the proportion of free throughfall could be higher than throughfall striking the canopy. However, in other locations the proportion of dripping throughfall could be higher, and it would have been affected more by fractionation processes due to interaction with a dryer canopy, increasing the isotopic differences between throughfall and rainfall. On the contrary, beyond 20 mm of rainfall, the homogenization of canopy saturation promotes the creation of canopy flow-paths that might reduce the residence time of water in the canopy and lead to a decrease in the isotopic differences between throughfall and rainfall.

Stemflow had more enriched $\delta^{18}O$ than throughfall, which is similar to results described by Kubota & Tsuboyama (2003), but the reasons for this remain unclear. Ikawa et al. (2011) highlighted how the isotopic composition of stemflow was strongly affected by the mixing of waters in the canopy and stems, with secondary effects of evaporation and isotopic exchange with ambient vapour. Although in our study most of the stemflow samples showed enriched $\delta^{18}O$, d-excess differences were not consistently negative. This suggests that, as proposed by Ikawa et al. (2011), evaporation, isotopic exchange, selection processes or a combination of all of them could affect the isotopic composition of stemflow. However, in contrast to throughfall, evaporation or isotopic exchange may have a greater impact on stemflow, as previous studies found that the residence time of water stored in branches and stems is longer than water stored in the canopy (Pypker, Levia, Staelens, & Van Stan, 2011). For Scots pine, Llorens & Gallart (2000) found that the specific storage capacity of stems was 6 times higher than for needles. Cayuela et al. (2018) found that, above 20 mm of rainfall, funnelling ratios for both species no longer increased. Above this threshold, stems funnelled water at their maximum capacity, reducing the exposure time of stored water to the atmosphere and reducing the effects of evaporation or isotopic exchange. In addition, stemflow in oaks had more negative d-excess values and 13% more enriched samples than pines. These differences between species could be related to the higher specific storage capacities of downy oak, which would enhance the impact of evaporation on their stems.

5.2. Spatial variability of the isotopic composition of throughfall and stemflow

At the intra-event scale, some spatial variability of the $\delta^{18}O$ was observed between throughfall collectors. This variability was higher for events with fewer than 20 mm of rainfall. Other studies that analysed the spatial variability of the isotopic composition of throughfall led to somewhat contradictory conclusions. Some of them observed an enrichment pattern due to the canopy cover (Brodersen et al., 2000; Kato et al., 2013), increasing the differences between throughfall and rainfall from the crown periphery to the crown centre. Other studies observed a lack of temporal stability in the spatial patterns of enrichment (Allen et al., 2014, 2015; Hsueh et al., 2016). Allen et al. (2014) related this lack of temporal stability with the existence of pre-event moisture retained in the canopy. But there is little justification of this process at our study site, as this is a plausible explanation only at very rainy and humid locations.

We found a positive relationship between the $\Delta\delta^{18}O_{TF\text{-}RF}$ and the canopy cover. This relationship was stronger for pines than for oaks. Greater canopy cover would imply a longer residence time of rain water moving through the leaves and stems in the canopy, which would increase the effect of fractionation processes. The lower spatial variability of the isotopic composition of throughfall associated with large rainfall events is probably related to the fact that for such events the canopy can easily reach saturation. On the contrary, for events of low magnitude, evaporation, isotopic exchange or canopy selective storage, along with a higher proportion of free throughfall, would have a greater impact on the spatial variability of the isotopic composition of throughfall, because of the unsaturated canopy. However, we observed that bulk samples were distributed mostly within throughfall mixing spaces, indicating that, at the event scale, the isotopic spatial variability of throughfall was in general lower than its isotopic temporal variability. A few exceptions were observed, possibly due to a higher effect of fractionation factors on some locations below the canopy during some rainfall events.

Stemflow also had marked isotopic variability for events of fewer than 20 mm of rainfall, suggesting that the isotopic modification of the stemflow was more variable between trees when their stems were not completely saturated and flow paths were not completely connected.

5.3. Rainfall intra-event isotopic modification

The greatest differences between $\delta^{18}O$ of throughfall and rainfall were observed at the beginning of the rainfall event, simultaneously with a decrease in d-excess. The greater isotopic enrichment in throughfall at the beginning of the event was consistent with a dryer atmosphere with high VPD, suggesting that evaporation in the canopy at this initial stage of rainfall could be important, as corroborated by higher interception losses. During the rainfall event, $\delta^{18}O$ differences between throughfall and rainfall tended to decrease, whereas d-excess tended to increase. Ikawa et al. (2011) suggested that differences between the $\delta^{18}O$ of throughfall and rainfall tended to disappear because the wetter the canopy becomes, the more flow paths are created, decreasing the lag time between rainfall and throughfall and reducing evaporation impact. In general, at the end of the event, throughfall had higher d-excess than rainfall, possibly because of the selection process. Therefore, the retention in the canopy of the final portion of rainfall, which usually had low d-excess values, would imply that throughfall measured during the final interval corresponded to rainfall lagged in earlier time intervals with higher d-excess. As observed by Kubota & Tsuboyama (2003), intra-storm isotopic trends in rainfall and throughfall may also vary depending on rainout effects or on changes in the origin of the vapour masses (Dansgaard, 1964). However, the general patterns observed in the three rainfall events analysed in detail suggest that evaporation, isotopic exchange or canopy selection are the drivers of the shift observed in the isotopic composition of rainfall when it passes through the canopy.

For large rainfall events, the activation of flow paths through the saturated canopy increases the amount of throughfall less affected by evaporation, equilibrium exchange or canopy selection, thus reducing the differences between throughfall and rainfall and resulting in an overlapping of the mixing spaces of throughfall and rainfall. On the contrary, for some small rainfall events, the final isotopic composition of throughfall is more greatly affected by fractionation factors. In this case, we speculate that temperature and relative humidity may have a big impact, leading to an enrichment in throughfall for high evaporation rates due to non-equilibrium fractionation. This process would be stronger in isotopically lighter rainfall events or it could lead to either depletion or enrichment during low evaporation rates due to equilibrium fractionation. These processes could explain why the mixing spaces of throughfall and rainfall for some events did not overlap and why sometimes the mixing space of throughfall was above or below the mixing space of rainfall.

6. CONCLUSIONS

This study showed that, though mean isotopic differences between rainfall, throughfall and stemflow can occur in both directions, there was greater throughfall enrichment at low air temperatures, and stemflow was more enriched than throughfall. Overall, no significant differences were found between species. Fractionation could be achieved by the mixture of factors previously described in the literature: evaporation, isotopic exchange and canopy selection processes. Although all processes probably occurred during the same rainfall event, evaporation seemed to have a higher impact at the beginning of rainfall. However, under low evaporation conditions, isotopic exchange may acquire more relevance. Fractionation caused by canopy selection processes appeared to be more important at the end of the event, when part of the final portion of rainfall was retained on the leaves and stems. All fractionation factors had a lower impact for events larger than 20 mm of rainfall because canopies were saturated and the lag time between rainfall, throughfall and stemflow was reduced.

Further research, to assess the movement of water through the canopy and to discern fractionation factors better, should consider an even higher temporal resolution of sampling collection for throughfall and stemflow. Measurement of the isotopic composition of the atmospheric vapour under the canopy could also shed light on possible enrichment or depletion under equilibrium conditions. Finally, complementary measurements like drop size distributions and velocities or stem flow velocities could help us to understand observed variations in the isotopic composition of throughfall and stemflow when compared with rainfall.

7. ACKNOWLEDGMENTS

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TABLE

Table 1. Meteorological characteristics of 3 events analysed at the intra-event temporal resolution in Figures 9 and 10 in the Scots pine and downy oak stand.

			Meteorological characteristics					Sequential samples		Spatial samples
Event	Date	Stand	Rainfall (mm)	Throughfall (mm)	Duration (hours)	Max Intensity in 30 min (mmh^{-1})	Total ET (mm)	Mean weighed rainfall $\delta^{18}O$ (‰) (mean ± SD)	Mean weighed throughfall δ18O (‰) (mean ± SD)	Mean bulk throughfall δ18O (‰) (mean ± SD)
8	31st of July	Pine	19.3	13.9	7.2	10.4	0.03	-4.41 ± 0.46	-4.94 ± 1.16	-4.83 ± 0.16
		Oak	21.7	14.0	7.2	9.5	0.08	-4.41 ± 0.69	-4.32 ± 0.60	-4.51 ± 0.18
10	15th of August	Pine	10.5	7.3	6.6	4.2	0.84	-12.02 ± 0.41	-10.80 ± 0.34	-10.81 ± 0.21
		Oak	13.9	9.3	6.6	5.3	0.85	-11.41 ± 0.36	-10.69 ± 0.95	-10.96 ± 0.18
12	3rd of September	Pine	53.8	46.1	25.1	7.6	0.71	-5.85 ± 0.89	-5.71 ± 0.99	-5.65 ± 0.35
		Oak	48.3	42.4	25.1	7.1	0.71	-5.84 ± 0.95	-5.48 ± 0.77	-5.79 ± 0.16

FIGURE CAPTIONS

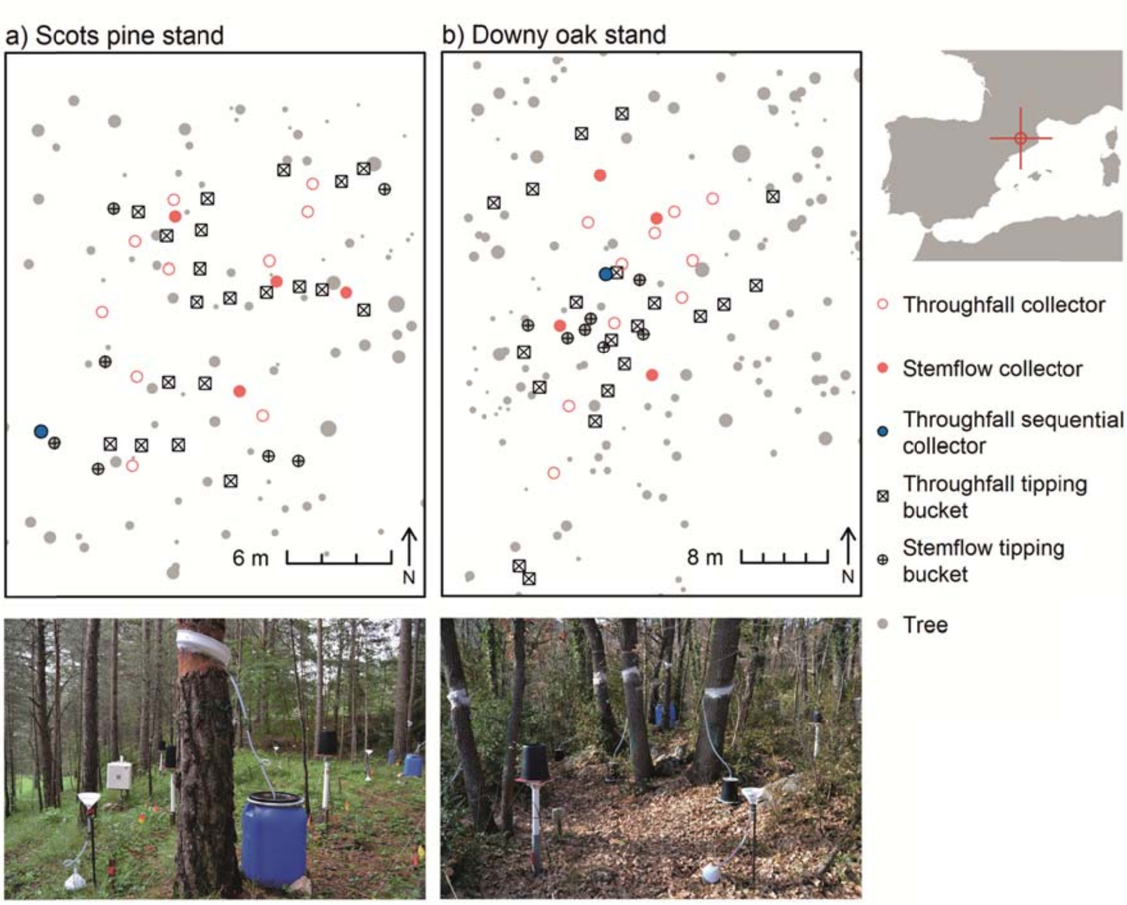

Figure 1. Location and maps of the monitored stands in the Vallcebre research catchments. (a) Scots pine stand and (b) Downy oak stand. Grey dots represent the distribution of trees. The size is proportional to the DBH.

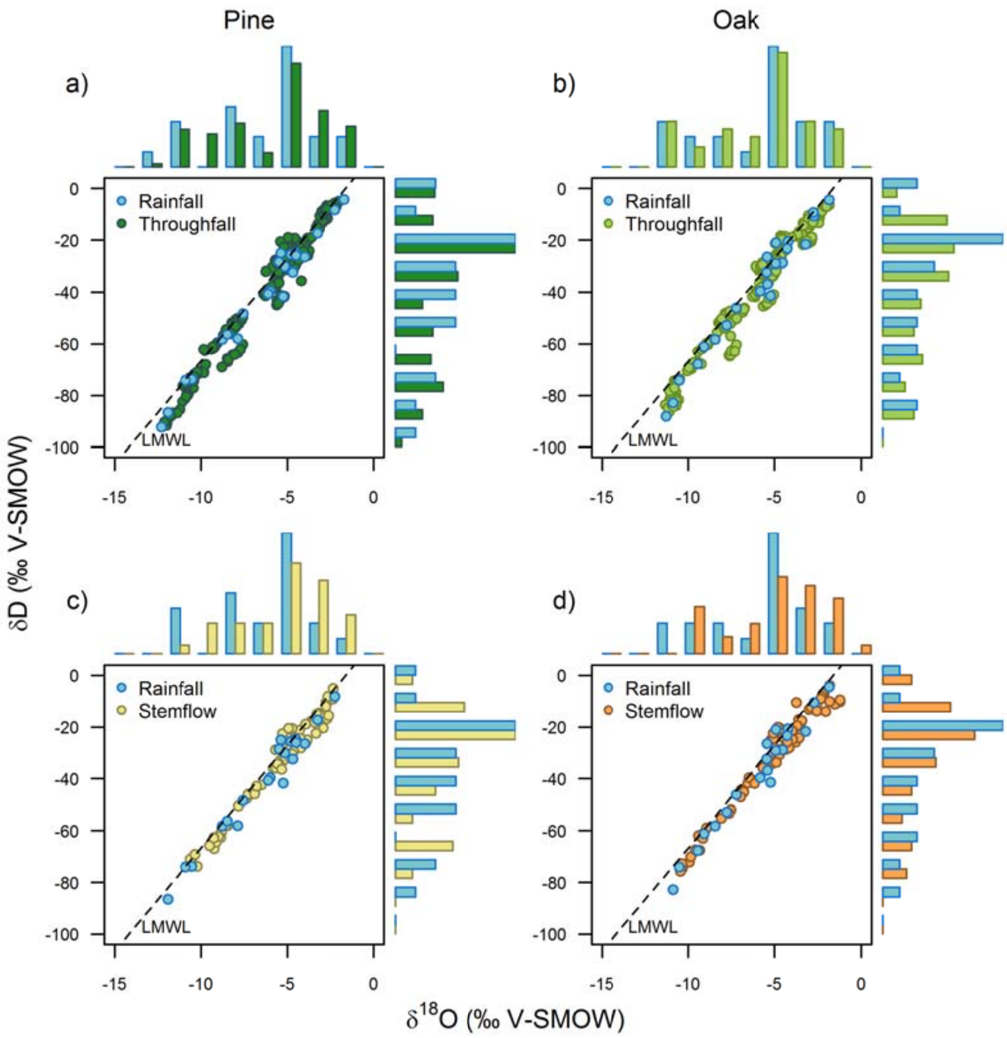

Figure 2. $\delta^{18}O$ and δD values of rainfall, throughfall and stemflow for the pines and oaks. The histogram borders show partitioning of the data sets at 10 equivalent intervals. The dashed line shows the local meteoric water line (LMWL). V-SMOW, Vienna-Standard Mean Ocean Water.

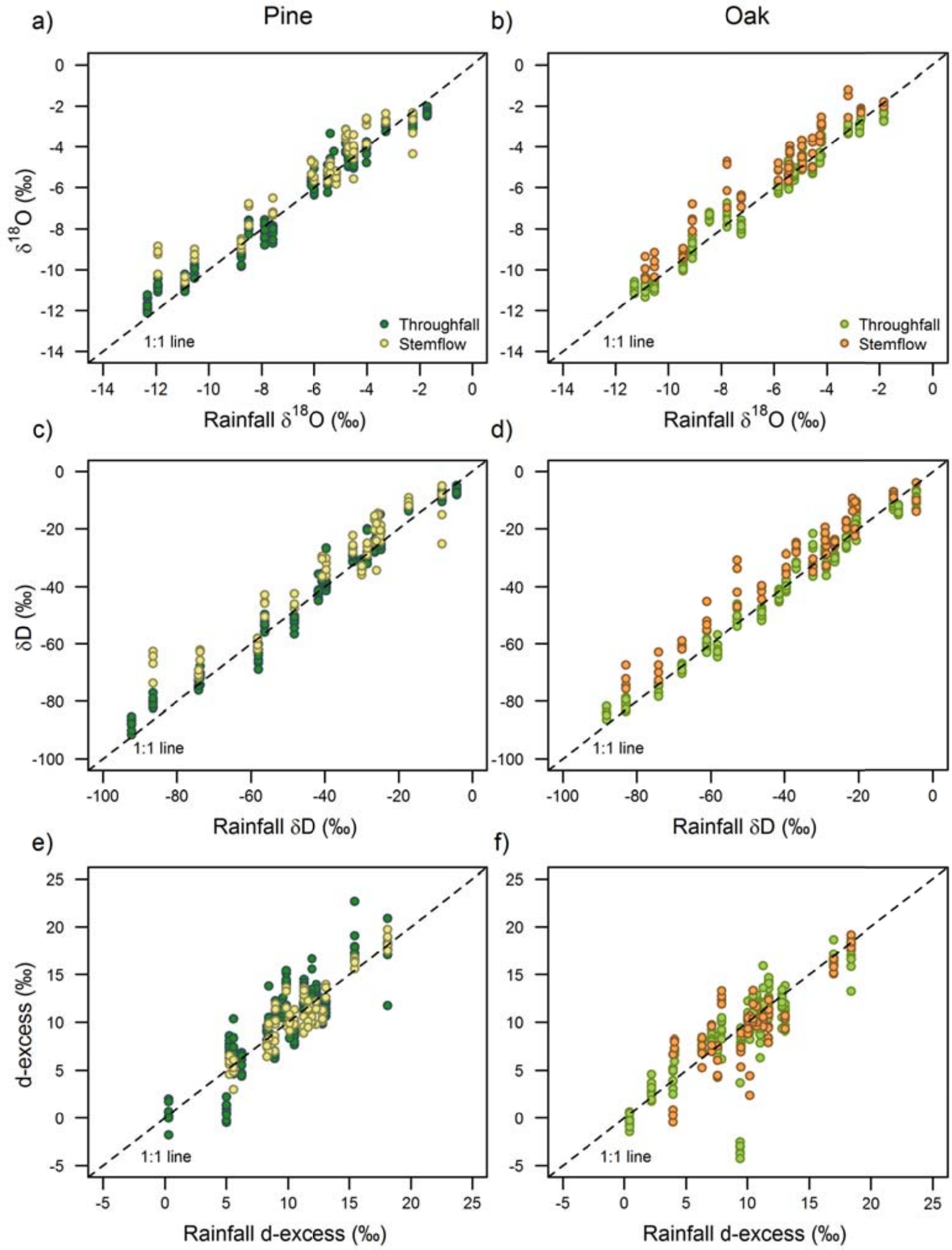

Figure 3. Relationship between $\delta^{18}O$ of rainfall and throughfall for the pines (a) and oaks (b), between δD of rainfall and throughfall (c, d) and between d-excess of rainfall and throughfall (e, f).

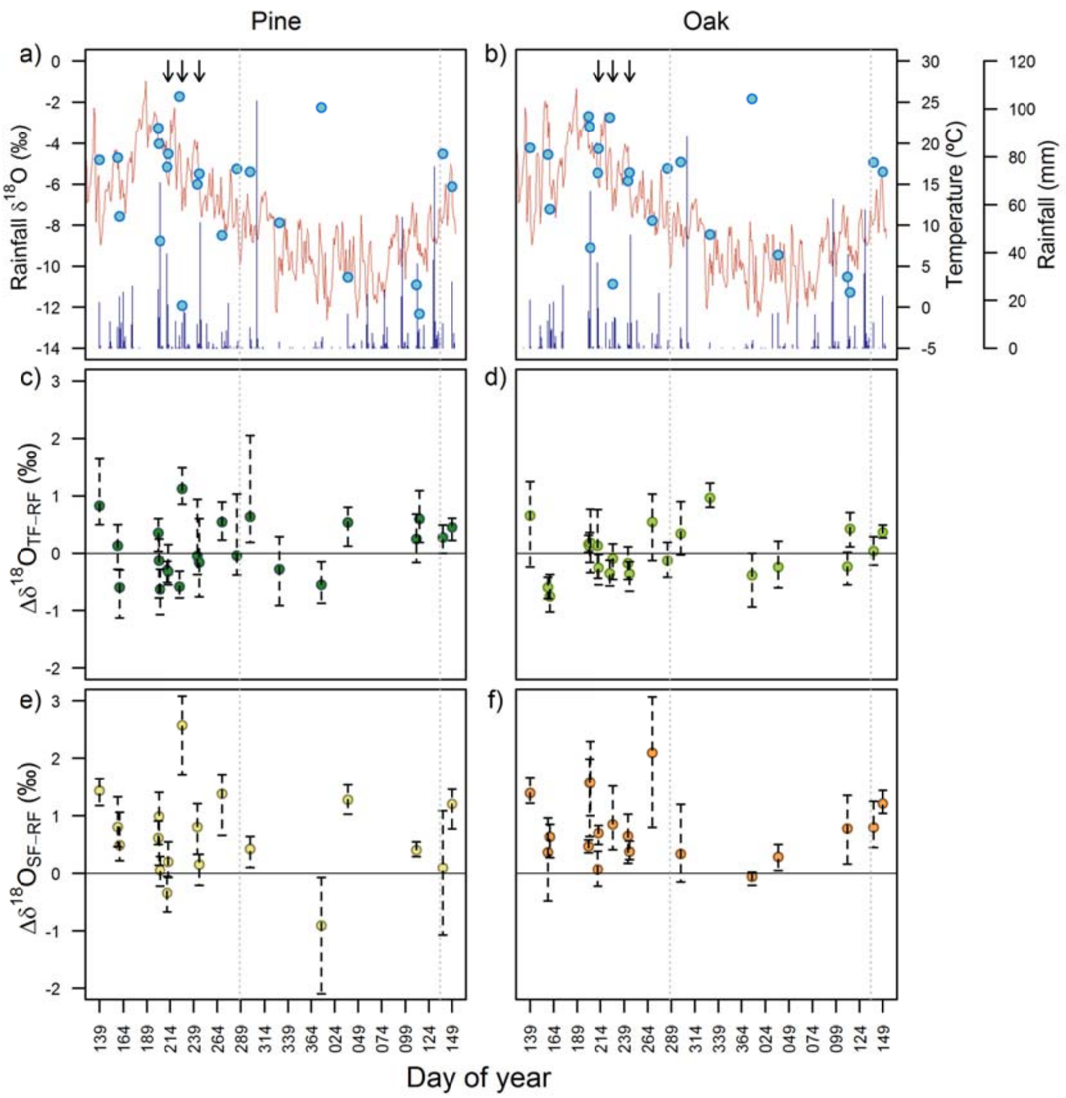

Figure 4. Time series of daily rainfall, daily temperature and event $\delta^{18}O$ of rainfall in the pine (a) and oak (b) stands. Time series of the isotopic $\delta^{18}O$ differences between throughfall and rainfall (c, d). Time series of the isotopic $\delta^{18}O$ differences between stemflow and rainfall (e, f). Black dashed lines represent the range of observed differences for each event (from 10 samples for throughfall and 4 samples for stemflow). Grey vertical dashed lines mark the growing and dormant seasons; and black arrows indicate the events analysed at the intra-event scale.

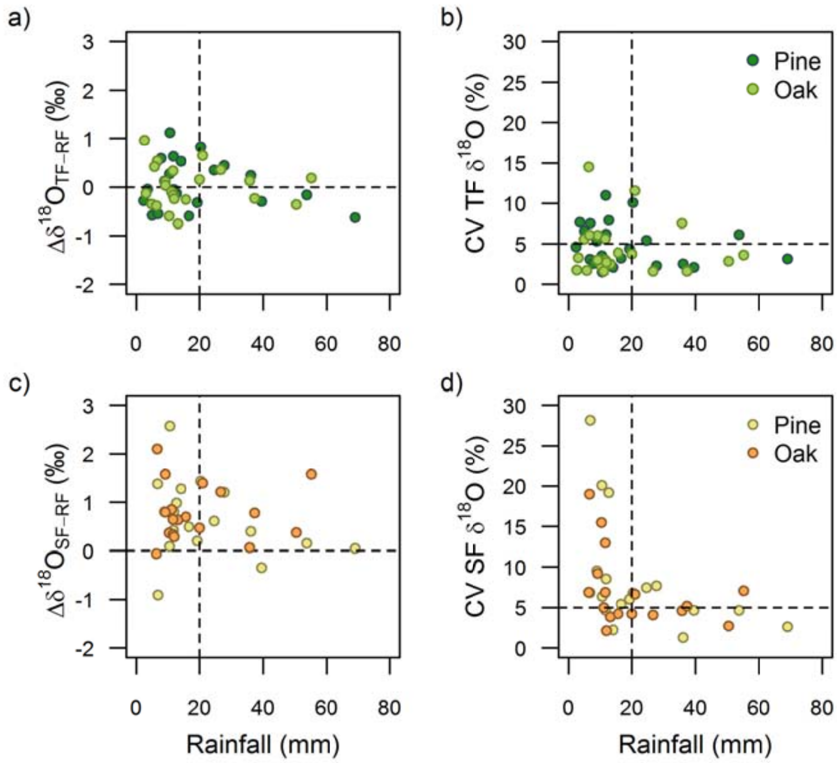

Figure 5. Mean event $\delta^{18}O$ differences between thoughfall and rainfall (a) and stemflow and rainfall (c) as a function of event rainfall depth. Spatial variability of $\delta^{18}O$ in throughfall (b) and stemflow (d), expressed as the coefficient of variation (CV) among collectors.

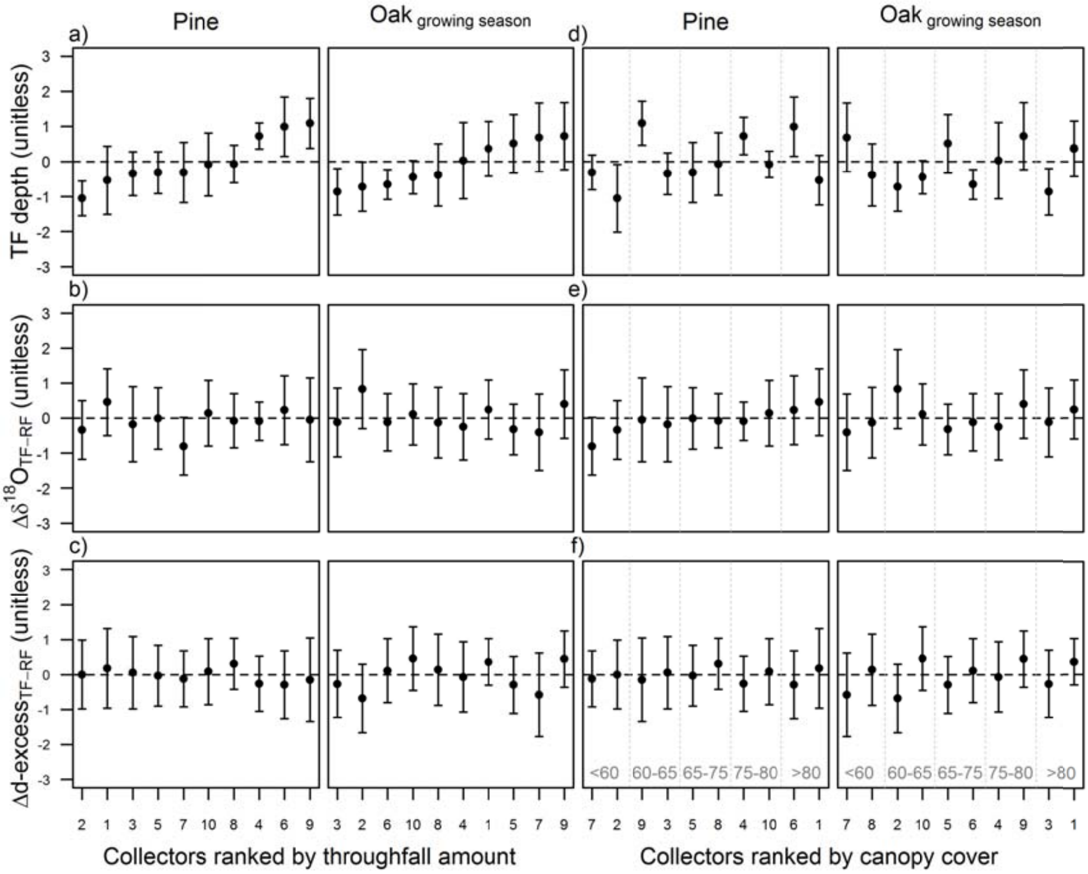

Figure 6. Time stability plots of normalized throughfall depth (a, d) in the pine (during the dormant and growing seasons) and oak (only during the growing season) stands; normalized $\delta^{18}O$ differences between throughfall and rainfall (b, e) and normalized d-excess differences between throughfall and rainfall (c, f). In (a, b, c) collectors are ranked according to throughfall amount; in (d, e, f) collectors are ranked by canopy cover. The grey number at the bottom of the Figure indicates the canopy cover percentage.

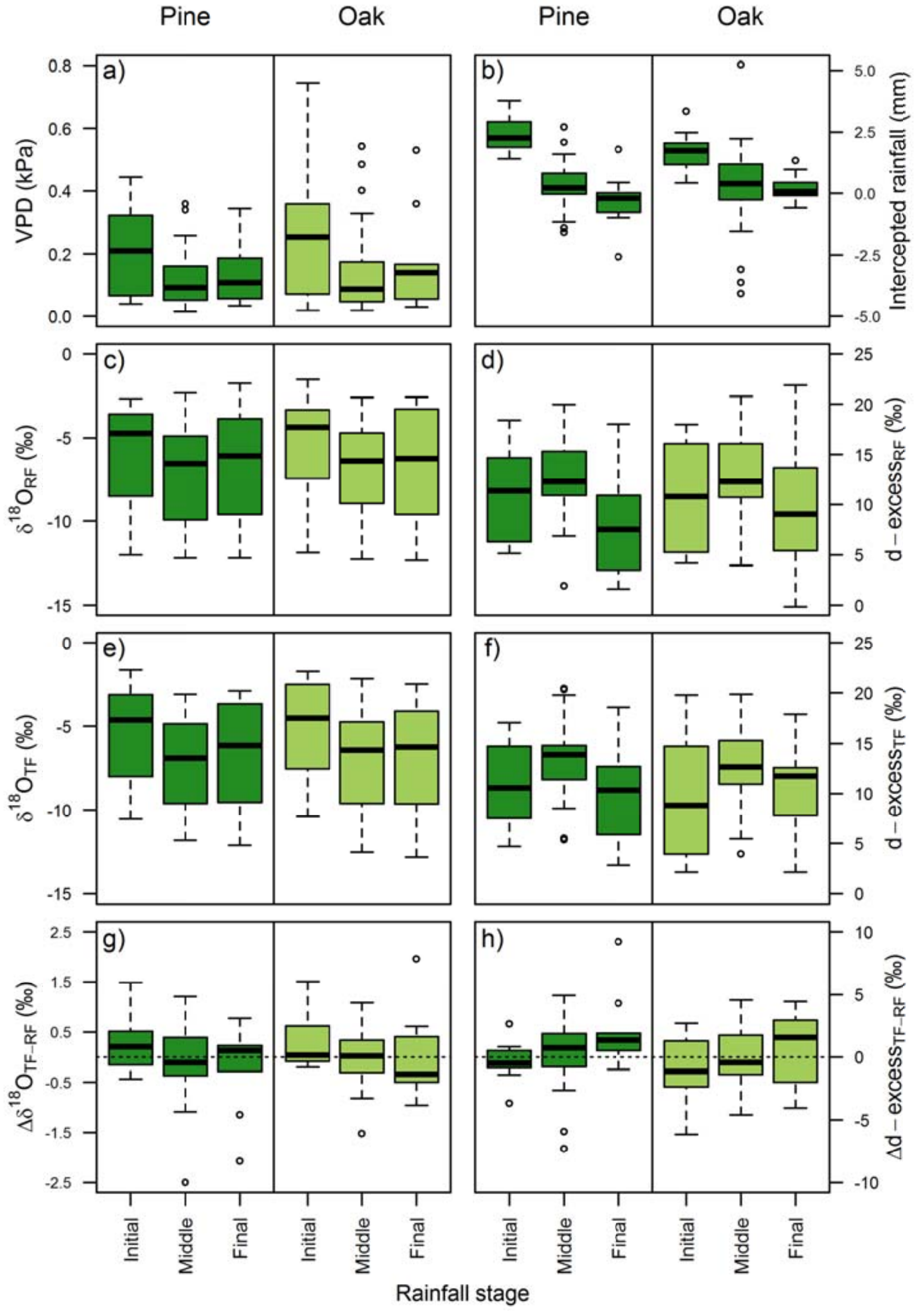

Figure 7. Boxplots of the intra-event dynamics of 10 rainfall events (> 10mm) in the pine and oak stands. VPD (a), Intercepted rainfall (b), rainfall $\delta^{18}O$ (c), rainfall d-excess (d), throughfall $\delta^{18}O$ (e), throughfall d-excess (f), $\delta^{18}O$ differences between throughfall and rainfall (g) and d-excess differences between throughfall and rainfall (h). Each boxplot represents a different phase (initial, middle and final) of the event. Initial represents the first 5 mm of each event; middle, all samples between the first and the last sample; and final, the last sample collected.

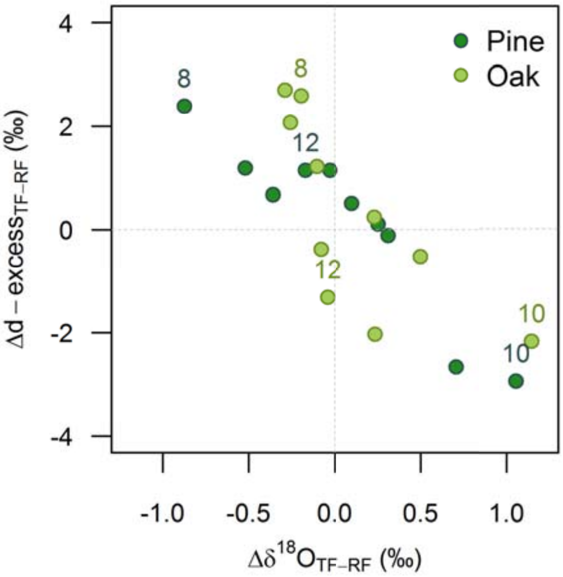

Figure 8. Relationship between the mean isotopic modification of throughfall ($\Delta\delta^{18}O_{TF-RF}$) and the change in d-excess (Δd-excess$_{TF-RF}$) for the sequentially analysed events in the pine and oak stands. Mean $\delta^{18}O$ and d-excess for each event are weighted by the volume of each sample. Numbers 8, 10 and 12 indicate the events analysed in detail in Figures 9 and 10.

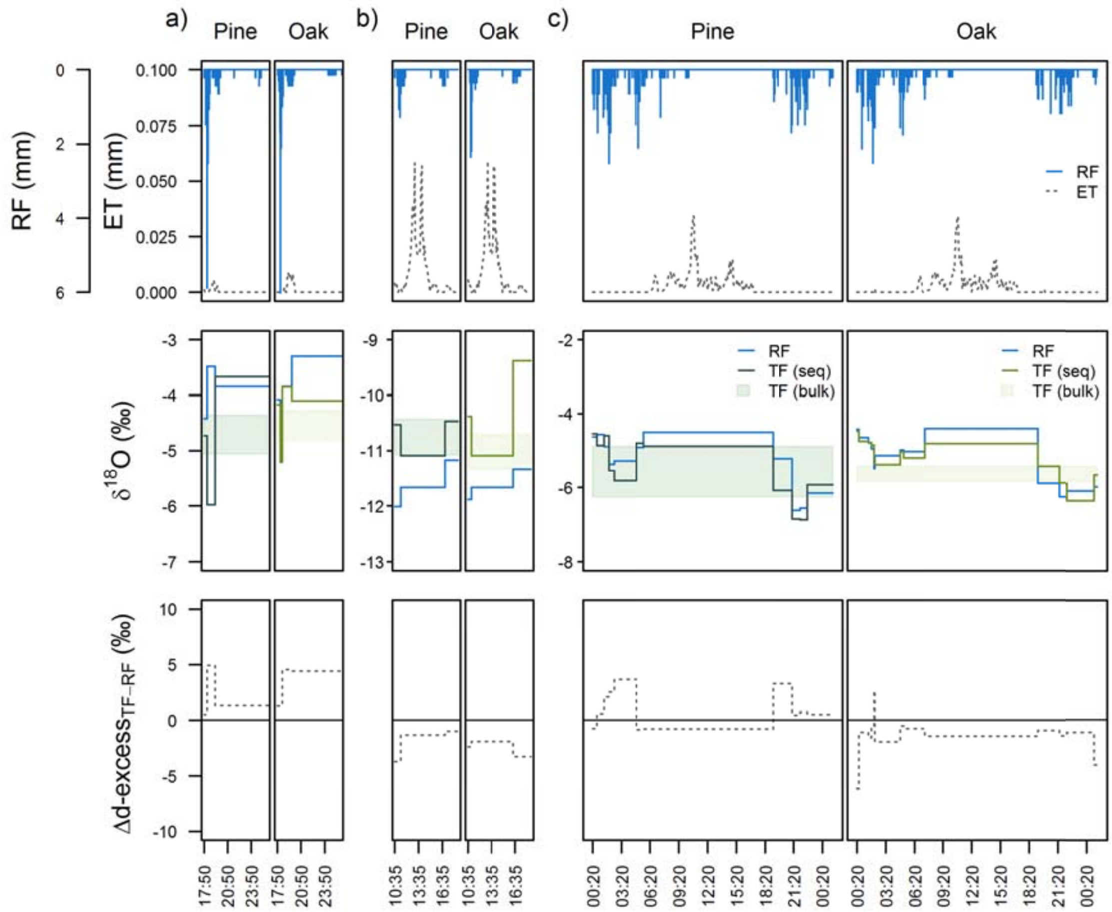

Figure 9. Comparison of the intra-event dynamics for three events: (a) Event 8 (July 31st), (b) Event 10 (August 15th) and (c) Event 12 (September 3rd). From top to bottom: time series of rainfall and wet canopy evaporation (5 min time step); $\delta^{18}O$ in rainfall and in throughfall collected by the sequential sampler (highlighted area represents the range of $\delta^{18}O$ of throughfall collected by the different bulk collectors in the forest plots); and d-excess difference between sequential throughfall and sequential rainfall samples.

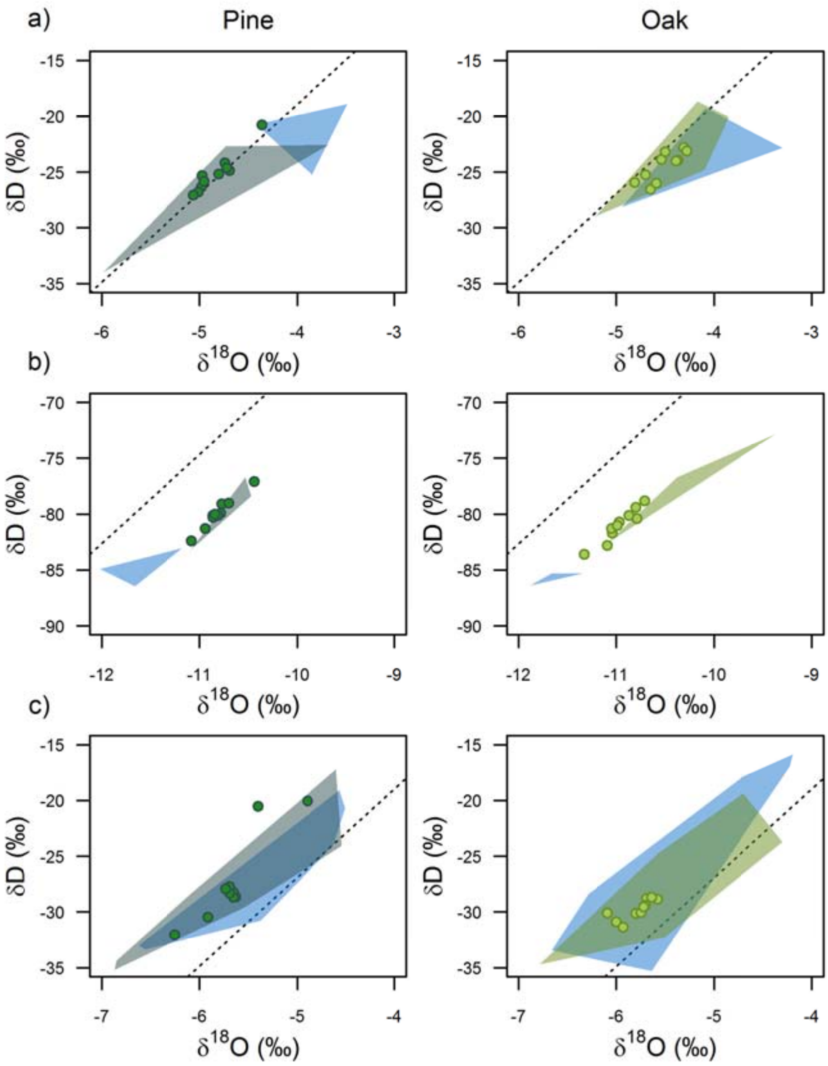

Figure 10. Dual plots for the rainfall events shown in Figure 9: (a) Event 8, (b) Event 10, and (c) Event 12, in the pine and oak stands. Dots represent bulk throughfall samples, blue areas represent the space created by the union of sequential rainfall samples and green areas (dark for pine and light for oak) represent the space created by sequential throughfall samples.